

(FILE 'HOME' ENTERED AT 08:25:30 ON 30 SEP 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 08:25:54 ON 30 SEP 2003

L1	3 S RFE AND LSG
L2	1 DUP REM L1 (2 DUPLICATES REMOVED)
L3	3 S WECA AND LSG?
L4	1 DUP REM L3 (2 DUPLICATES REMOVED)
L5	0 S LSGG AND RFE
L6	4 S RFE AND LIPOOLIGOSACCHARIDE
L7	2 DUP REM L6 (2 DUPLICATES REMOVED)

	Typ	Hits	Search Text	DBs
1	IS&R	6	((("4703008") or ("4745051")).PN.	USPAT; EPO; JPO; DERWENT;
2	BRS	0	glycotransferase near5 oligosaccharide	USPAT; EPO; JPO; DERWENT;
3	BRS	0	glycotransferase near5 oligosaccharide	USPAT; EPO; JPO; DERWENT;
4	BRS	0	glycotransferase near10 oligosaccharide	USPAT; EPO; JPO; DERWENT;
5	BRS	33	glycotransferase	USPAT; EPO; JPO; DERWENT;
6	IS&R	18	((("5747326") or ("5736533") or ("5705367") or ("5308460") or ("5370872") or ("5945322") or ("5945322").PN.	USPAT; EPO; JPO; DERWENT; IBM_TDB
7	BRS	0	heptose near5 haemophilus	USPAT; EPO; JPO; DERWENT;
8	BRS	0	heptose near5 influenzae	USPAT; EPO; JPO; DERWENT;
9	BRS	0	heptose near10 influenzae	USPAT; EPO; JPO; DERWENT;
10	BRS	13	heptose and influenzae	USPAT; EPO; JPO; DERWENT;
11	BRS	1792	Haemophilus near1 influenzae	USPAT; EPO; JPO; DERWENT;
12	BRS	12	(Haemophilus near1 influenzae) and heptose	USPAT; EPO; JPO; DERWENT;
13	IS&R	3	("5830742").PN.	USPAT; EPO; JPO; DERWENT;
14	BRS	0	glycosyltransferase and heptose and galactose	USPAT; US-PGPUB; EPO; JPO; DERWENT;
15	BRS	0	glycosyltransferase and heptose	USPAT; US-PGPUB; EPO; JPO; DERWENT;
16	BRS	5	glycosyltransferase and heptose and galactose	USPAT; US-PGPUB; EPO; JPO; DERWENT;
17	BRS	2	lsg and rfe	USPAT; US-PGPUB; EPO; JPO; DERWENT;
18	BRS	0	lsg and weca	USPAT; US-PGPUB; EPO; JPO; DERWENT;
19	BRS	29	rfe and los	USPAT; US-PGPUB; EPO; JPO; DERWENT;

	Time Stamp	Comments	Error Definition	Errors
1	2003/09/30 06:39			0
2	2001/04/06 08:14			0
3	2001/04/06 08:14			0
4	2001/04/06 08:14			0
5	2001/04/06 08:15			0
6	2001/04/06 08:42			0
7	2001/04/06 11:24			0
8	2001/04/06 11:25			0
9	2001/04/06 11:25			0
10	2001/04/06 11:25			0
11	2001/04/06 11:25			0
12	2001/04/06 11:25			0
13	2001/04/09 10:27			0
14	2001/05/04 11:03			0
15	2001/05/04 11:03			0
16	2001/05/04 11:03			0
17	2003/04/28 13:32			0
18	2003/04/28 13:32			0
19	2003/04/28 13:32			0

	Type	Hits	Search Text	DBs
20	BRS	2	lsg and rfe	USPAT; US-PGPUB; EPO; JPO; DERWENT;
21	BRS	0	lsgg and rfe	USPAT; US-PGPUB; EPO; JPO; DERWENT;
22	BRS	296	lsg	USPAT; US-PGPUB; EPO; JPO; DERWENT;
23	BRS	327	rfe	USPAT; US-PGPUB; EPO; JPO; DERWENT;
24	BRS	1	lipooligosaccharide near2 synthesis near2 gene	USPAT; US-PGPUB; EPO; JPO; DERWENT;
25	BRS	0	(lipooligosaccharide near2 synthesis near2 gene) and rfe	USPAT; US-PGPUB; EPO; JPO; DERWENT;
26	BRS	5	rfe and lipooligosaccharide	USPAT; US-PGPUB; EPO; JPO; DERWENT;
27	BRS	44	rfe and regulated	USPAT; US-PGPUB; EPO; JPO; DERWENT;
28	BRS	0	weca and lsg	USPAT; US-PGPUB; EPO; JPO; DERWENT;
29	BRS	2	rfe and lsg	USPAT; US-PGPUB; EPO; JPO; DERWENT;
30	BRS	10	weca	USPAT; US-PGPUB; EPO; JPO; DERWENT;
31	BRS	14023	reeves	USPAT; US-PGPUB; EPO; JPO; DERWENT;
32	BRS	1	reeves and weca	USPAT; US-PGPUB; EPO; JPO; DERWENT;

	Time Stamp	Comments	Error D	finite Errors
20	2003/09/30 06:41			0
21	2003/09/30 06:41			0
22	2003/09/30 06:41			0
23	2003/09/30 06:42			0
24	2003/09/30 06:42			0
25	2003/09/30 06:42			0
26	2003/09/30 06:45			0
27	2003/09/30 08:24			0
28	2003/09/30 08:26			0
29	2003/09/30 08:24			0
30	2003/09/30 08:27			0
31	2003/09/30 08:27			0
32	2003/09/30 08:27			0

ngly, the genes encoding the remaining enzymes in this pathway are located upstream and somewhat removed from the *psbI* gene (*psbABDE*).

[0309] *psbJ*.

[0310] The distance between *psbI* and *psbJ* is 17 bp. A putative RBS is present immediately following the stop codon of *psbI*, 13 bp from the AUG start codon of *psbJ* (FIG. 4). *PsbJ* demonstrates reasonable homology to Bp1E (52.6%) of *B. pertussis*, a glycosyl transferase thought to attach either 2,3-diNAcManA or FucNAcMe to the O-unit (Allen and Maskell, 1996) (Table 2). *TrsE* of *Yersinia enterocolitica* also has homology to *PsbJ* (Skurnik et al., 1995), and is thought to be one of the galactosyl- or mannosyl transferases. An alignment of *PsbJ* and *PsbJ*-like proteins is shown in FIG. 39. As Bp1E also has limited homology with *PsbH*, it is likely that both *PsbH* and *PsbJ* are the transferases involved in the addition of the two mannuronic acid residues to the B-band O-antigen unit. *PsbJ* has two putative membrane-spanning domains at the N-terminus, and may be anchored in the cytoplasmic membrane.

[0311] *psbK*.

[0312] The start codon of *psbK* overlaps the stop codon of *psbJ*, and the second codon is AAA (FIG. 32). *PsbK* demonstrates homology to a series of glucose dehydratases, including StrP of *Streptomyces glauciens* involved in streptomycin biosynthesis (accession number 629223), ExoB of *R. meliloti* (Buendia et al., 1991), ORF o355 (incorrectly assigned RffE) of *E. coli* (Daniels et al, 1992, Macpherson et al., 1994), GraE of *Streptomyces violaceorubens* (Bechtold et al., 1995) and RfbB of a number of organisms including *N. meningitidis* (Hamerschmidt et al., 1994) and *E. coli* (Marolda and Valvano, 1995). Alignment of these proteins show the presence of an NAD-binding domain (GXXGXXG) near the N-terminal end (FIG. 5; Macpherson et al., 1994). RfbB and o355 are known to be involved in the biosynthesis of FucNAc (Meier-Dieter et al., 1992). Based on these homologies, *PsbK* is thought to be dTDP-D-glucose 4,6-dehydratase, required as the second step in the biosynthesis of FucNAc.

[0313] *psbL*.

[0314] There are 59 bp between the end of *psbK* and the start of *psbL* but no RBS could be detected in the region preceding the double start codons (FIG. 32). Identification of the *psbL(rfbA)* gene has previously been reported (Dasgupta

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Role of the rfe gene in the biosynthesis of the Escherichia coli O7- specific lipopolysaccharide and other O-specific polysaccharides containing N-acetylglucosamine

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We report that rfe mutants of wild-type strains of Escherichia coli O7, O18, O75, and O111 did not express O-specific polysaccharide unless the rfe mutation was complemented by a cloned rfe gene supplied in a plasmid. The O polysaccharides in these strains are known to have N- acetylglucosamine (GlcNAc) in their O repeats. In addition, in vitro transferase assays with bacterial membranes from either the O7 wild- type strain or its isogenic rfe mutant showed that GlcNAc is the first carbohydrate added onto the lipid acceptor in the assembly of the O7 repeat and that this function is inhibited by tunicamycin. Our results indicate that the rfe gene product is a general requirement for the synthesis of O polysaccharides containing GlcNAc.

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